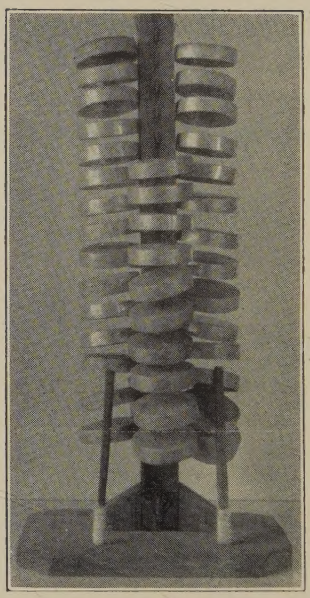
Kentucky
Agricultural Experiment Station
University of Kentucky

1. Do Tobacco Plants Recover from, and Develop Immunity to Ringspot?
2. Susceptibility of Tobacco Plants Visibly Affected with Mild Tobacco Mosaic to Other Strains of the Virus.
3. Localization and Resistance to Tobacco Mosaic, in Nicotiana.

BULLETIN NO. 360
(RESEARCH BULLETIN)

Lexington, Ky.
December, 1935
(179)



Home-made stand with dishes and swabs used in plant virus inoculations. The aluminum milk dishes are 2½ inches in diameter by ⅝ inch deep. The stand is made of wood and may be varied to fit any type of autoclave. The dishes cool quickly, and have a sharp enough edge so that the piece of leaf to be used as inoculum may be readily cut off, with the aid of the cloth swab pictured, or a glass spatula. The dishes are sufficiently rigid so that the inoculum may be crushed in them after adding a small volume of water. The sterile side of the dish or a paper or card may be used to support the leaf while inoculating.

BULLETIN NO. 360
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1. Do Tobacco Plants Recover From and Develop Immunity to Ringspot?

By W. D. VALLEAU

One of the outstanding examples of supposed recovery from a virus disease in plants and development of an acquired immunity is that which occurs in tobacco following an "acute" attack of the ringspot disease (5). Other instances in which a plant infected with a given virus did not recover but appeared to be immune to another strain of the same kind of virus have been similarly interpreted as acquired immunity. Kunkel concluded that "the principles involved are the same as those made use of in human and animal pathology for immunization by vaccination" (3). Chester (1), in his extensive review of the literature on acquired immunity, concluded from experiments of this type that "acquirement of immunity to virus (by plants) is a fact," and "the immunity thus acquired is closely bound up in the living cell, not freely diffusible." The evidence presented so far, seems to justify a different hypothesis to explain what has seemed to be recovery from ringspot and the acquirement of immunity in plants affected by this and other viruses—one which assumes neither recovery nor the acquirement of immunity, in the sense in which Chester has defined it.

The facts with respect to ringspot are, briefly, as follows: If a tobacco leaf is inoculated with the ringspot virus, the virus multiplies and produces necrotic rings about the points of infection. The virus continues to multiply, enters the main stem of the plant and is carried upward into the growing point and downward into the roots; then, as the younger leaves expand,

ring and line patterns typical of the disease develop on some of them, the number of affected leaves depending on the vigor of the plant. All leaves that develop subsequently from this growing point are without patterns, altho highly viruliferous; the plant is then said to have recovered. If the apparently healthy leaves are then inocualted with the ringspot virus, there is no evident result from the inoculation. In the words of Price (5), "tobacco plants normally recover from ring-spot and acquire an immunity to this disease."

The question as to whether the ringspot patterns constitute the only symptom of the disease, and whether the plant can be considered to have recovered when patterns are no longer produced, is one of paramount importance to an understanding of this virus disease. There are several reasons for believing that the ring patterns are but one, and frequently a minor, symptom of the disease. The ring pattern, on inoculated leaves is common to several virus diseases of tobacco, including certain strains of tobacco mosaic, cucumber mosaics, the etch virus diseases, the healthy-potato virus, and ringspot. In ringspot the patterns occur on the inoculated leaves first; then, as the virus is carried upward, necrotic rings, spots, and line patterns develop irregularly in one leaf after another as the leaves expand, until finally the so-called oak-leaf patterns develop on one or more leaves, these leaves are followed by leaves without patterns, and the plant appears to have recovered. It seems that the leaves bearing the nearly symmetrical oak-leaf patterns were very small at the time the growing point was invaded by the virus. Leaves developed subsequently presumably are invaded uniformly by the virus as they develop, and for this reason are free from patterns. The theory that invasion of healthy tissue by the virus causes ring spotting is supported by the fact that slow-growing plants or very young plants with few growing-point leaves pass thru the ringspot stage with few patterned leaves, whereas very vigorous plants produce many patterned leaves, and require a much longer time to reach the patternless stage. The theory is also supported by evidence obtained by the writer in a study of the mosaic-resistant Ambalema variety of

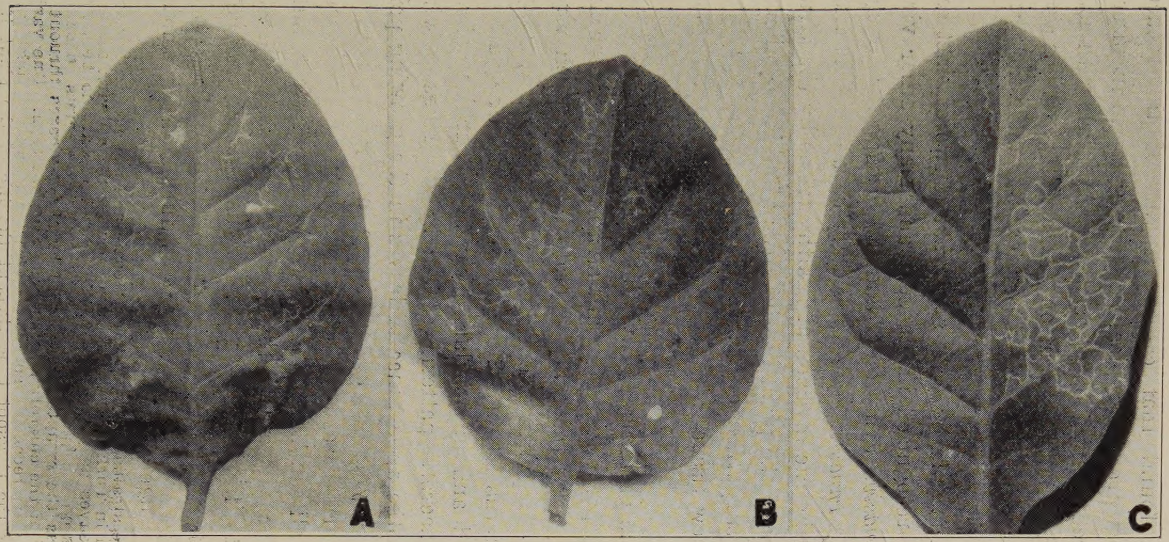

Figure 1. A and B. Necrotic oak-leaf patterns produced in uninoculated invaded leaves of Turkish tobacco by two distinct strains of yellow tobacco mosaic (11022 and 11025). The patterns appeared about 16 days after inoculation and 10 days after growing-point symptoms appeared. Similar patterns were produced by several other strains of tobacco mosaic and by inoculation of tobacco plants with dried tobacco 52 years old, which eliminates the possibility that the ringspot virus may be concerned. C. Necrotic ring patterns on an inoculated half of a leaf of an F_1 hybrid of Ambalema with a white Burley, inoculated with a green mosaic variant of white mosaic. Two other tobacco mosaic viruses caused similar ring patterns in the F_1 hybrid. Photo May 1, 1935. Patterns of this type have developed only following periods when the greenhouse temperature dropt below 50° F. during the period of invasion.

tobacco. It was found that certain strains of the tobacco mosaic virus which produce typical mosaic symptoms in susceptible varieties, produce ring patterns nearly identical with some of those produced by ringspot, but only after the virus has slowly invaded expanded leaves. The virus does not enter the growing-point leaves.

The problem may be clarified somewhat if two distinct sets of symptoms are recognized in ringspot and many other virus diseases: (1) *invasive* symptoms, and (2) *occupative* or systemic symptoms. *Invasive* symptoms may be defined as those symptoms caused by the invasion of healthy tissue by the virus; as the penetration of the plant by the virus after inoculation of a lower leaf. *Occupative,* or systematic, symptoms are those which occur in new tissues after invasion of the growing point has occurred.

Ring and line patterns, as invasive symptoms, are not confined to the ringspot virus. Certain strains of cucumber and tobacco mosaic viruses, the healthy-potato virus, the etch virus, and the vein-banding virus may produce necrotic spots, stipple necrotic line patterns (Figure 2), or solid necrotic line patterns on the inoculated and uninoculated leaves of Turkish tobacco very similar to necrotic patterns caused by the ringspot virus (Figure 1). On the appearance of leaves in the growing point which bear typical symptoms of the disease in question, the ringspot type of pattern ceases to appear.[1] The rinspot symptom is therefore not at all confined to the tobacco ringspot virus, but is produced by other viruses as they invade masses of healthy tissue. Ringspot differs from the other virus diseases mentioned in that it requires a longer time for invasion of the growing point, it produces more severe symptoms during its invasion of

[1] There are some strains of the tobacco mosaic and the cucumber mosaic viruses which, even in Turkish tobacco, never occupy the growing point but invade the young leaves as they develop. The virus appears to be carried at random to points on the leaf and to spread from these points producing ring patterns. Thus the symptoms produced by these viruses thruout the life of the plant are of the chlorotic, or necrotic ringspot type. One variant of yellow mosaic was recently isolated from Ambalema, which causes necrotic patterns of the ringspot type thruout the life of the plant if the plant is not vigorous, whereas in vigorous plants necrotic ringspot patterns are produced in invaded leaves and typical, very rugose mosaic symptoms in the growing-point leaves.

normal leaves, and it differs in the type of symptom produced following its occupation of the growing point. If ringspot patterns are in fact invasive symptoms, the patterns in the growing point of ringspot plants may be put into a category with those produced on inoculated leaves, the true symptoms of the dis-

Figure 2. F_1 Burley 5 x Ambalema, inoculated with white mosaic. Necrotic spotting is shown on the small inoculated leaf at the right. Necrosis of the stipple necrosis type is shown on the invaded leaf at the left. The tip of this leaf and the older leaf at the lower right were older tissue and were not invaded. The growing-point leaf shows typical mosaic symptoms. The tip of this plant might be considered to be recovering from the necrotic stage of the disease in the same way that ringspot plants recover from the necrotic stage of ringspot.

ease, as in other virus diseases, not occurring until after invasion of the growing point has been accomplished. Another disease whose most characteristic symptoms are produced soon after inoculation, is tomato streak. Necrosis of leaf and stem tissue is an early symptom of streak and is very evidently due to invasion of healthy tissue. Later, streak plants "recover", and

the symptoms are those of mosaic. There are many other virus diseases in which the early, or invasive, symptoms are strikingly different from the later symptoms. Mosaic burning is caused by the invasion of healthy tissue by a burning strain of the tobacco mosaic virus.

The second reason for believing that ringspot plants do not recover is afforded by the studies of Price (5), in which he showed that the upper leaves of ringspot plants are darker colored, and that the plant makes slower growth thruout life than

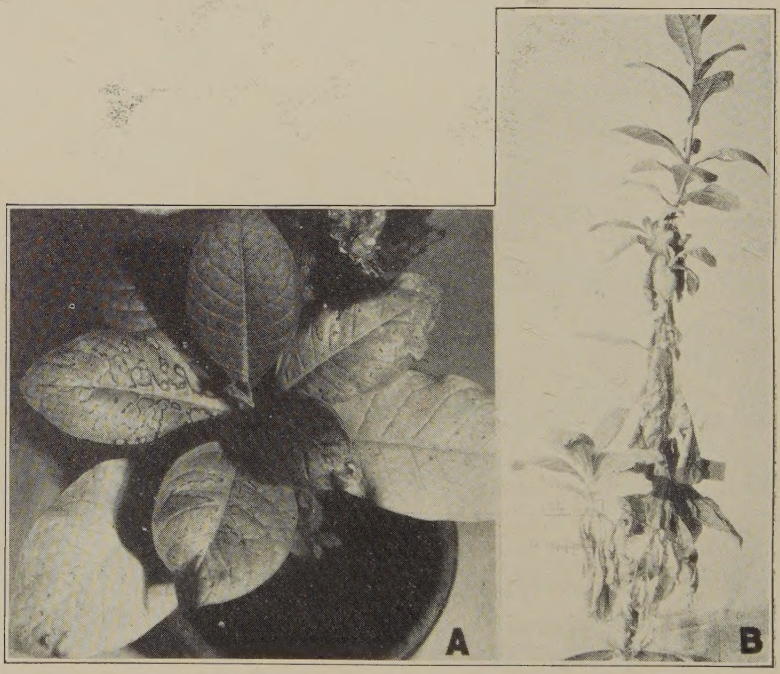

Figure 3. Ringspot patterns produced on a tertiary shoot of a plant of Kentucky White Burley No. 5, 172 days after inoculation with the green ringspot virus. B. The plant as it appeared a few days after the appearance of patterns. The original inoculated leaf is marked by a paper label. Dead leaves bearing necrotic patterns may be seen above this leaf. This shoot was a lateral produced after cutting back the original plant to about 8 inches. The tertiary shoot on the left of the original stalk developed necrotic patterns long after the period of so-called recovery. A. A detail photo of the shoot which developed patterns. The twelfth leaf on this shoot was the first to show patterns. It is probable that the virus did not enter this shoot until after it had made considerable growth. It may simply represent a case of slow invasion of stalk tissue. One leaf inoculated October 21, 1934, patterns on tertiary shoot April 21, 1935, photographed May 1, 1935.

a healthy plant. He and others, have shown that the patternless leaves carry the virus in a rather high concentration, which indicates that the virus enters the cells of the patternless leaves, and multiplies in them in the same way as in the patterned leaves.

The third reason is that ringspot-affected plants can frequently be identified in the field when no patterns can be seen, by the peculiar chlorosis or necrosis of the edges of leaves of lateral shoots, or "suckers." This follows a period of cool weather when the temperature has fallen to about 50° F. (7). The same symptom develops in "recovered" yellow and green ringspot plants growing in the greenhouse during cool periods. This symptom alone should be sufficient to convince one that green ringspot plants do not recover. Furthermore, affected plants which have passed thru the ringspot stage of the disease may again develop leaves with extensive ring and line patterns. These commonly develop in the leaves of lateral shoots produced on stubble of ringspot plants following harvest, long after the time of the so-called recovery, and sometimes on plants in the greenhouse which have been cut back after the plants have been developing patternless leaves for some time (Figure 3). That the patterns do not necessarily develop on leaves which were differentiated at the time of initial infection is proved by an instance already reported (7) of necrotic ring patterns on leaves of a tertiary bud of a ringspot plant affected by seed-borne ringspot virus, which had been cut back twice. The plant was over a year old when the first rings developed, but was viruliferous thruout this period, as proved by frequent transfers of the virus to healthy plants, in which typical ringspot patterns resulted. It seems possible that lateral buds may sometimes remain free from the virus until growth commences, when infection of the bud occurs, and patterns develop as in a newly inoculated plant.

The fourth and perhaps best reason for believing that ringspot plants do not recover is afforded by the yellowing strain of the ringspot virus described by Valleau (7). This virus appears to be of the same kind as the ordinary strain of the virus studied at the Virginia Experiment Station and used by Price in his

studies, for the following reasons: The symptoms on inoculated leaves are identical, as are also the symptoms which develop in the growing-point leaves, except that the yellowing strain causes the chlorotic parts of the patterns to become yellow, instead of light green of the ordinary strain. Both viruses cause leaf-edge chlorosis and necrosis and both cause an appreciable amount of of pollen abortion, a condition not known to be caused by any other tobacco virus. The green strain of the virus gives protection against infection with the yellow strain on leaves produced following so-called recovery (tests by the writer) just as it protects against reinfection by the green strain itself. However, neither strain gives complete protection against itself or the other strain in the invaded portions of the plant—that is, a leaf in the invaded zone of a plant where ring patterns have been produced may be reinoculated, with the production of local symptoms. This means that patterned leaves are only partially invaded by the virus, and that large areas are still virus-free. Finally, Henderson (2) demonstrated that the Virginia strain of the ringspot virus was carried in petunia seed, and Valleau showed that a similar green strain was sometimes carried in tobacco seed, while the yellowing strain was carried in tobacco seed sometimes to the extent of 15 percent.[2] If it is admitted that the yellowing virus is of the same kind as the ordinary strain, then the conclusion seems inevitable that ringspot plants do not recover following the so-called acute stage, for the leaves of plants affected by the yellowing strain, altho dark green at first, gradually bleach to a light yellowish green, giving the plant a strikingly abnormal appearance. Seedlings affected by the seed-borne yellowing strain, while green at first, bleach in a few days and remain so thruout life, but do not develop ring patterns. These plants could never be considered healthy. Bleaching also occurs in *Solanum tuberosum* and *S. carolinense* plants affected with ringspot. As plants affected by the ordinary ringspot virus do not grow normally following so-called recovery, and as those affected by the yellowing strain are obvious-

[2] Seed containing the yellow strain was sent to Dr. H. H. Thornberry, then of the Rockfeller Institute, Princeton, N. J., in 1932. See Phytopathology 25:938, 1935. He had no difficulty in recognizing the yellow ringspot seedlings and transferring the virus to healthy plants.

ly diseased thruout life, it cannot be concluded logically that ringspot plants which no longer develop patterns have recovered from the disease. If the cell were taken as the unit, it is doubtful if the question of recovery would ever have been raised.

Chester (1) has defined acquired physiological immunity as "the capability of withstanding infection acquired by the host either through the introduction of protective chemical substances of biological origin (passive) or through the elaboration of such protective substances in the host as a result of stimulation by the parasite." If we accept the proposition that the development of ring patterns occurs as the result of the invasion of masses of healthy tissue by the virus, and that the plant is still diseased following the production of patternless leaves, then the problem of immunity does not seem to enter into the failure of a second inoculation to produce rings. The cells of the patternless leaves are already affected to the limit by the virus, and the addition of a few more units of the virus to an occasional cell cannot be expected to produce an effect.

If now we consider the protective effect of one strain of a virus against a more injurious strain, it would seem that exactly the same phenomenon is concerned as in the case of ringspot. There appears to be no fundamental difference in what takes place in a ringspot plant and a mosaic plant after invasion of the growing point. Complete invasion of the growing point by the ringspot virus is followed by leaves which are without patterns, whereas uneven and incomplete invasion of the young leaves of mosaic plants results in mosaic patterns. If invasion of leaves in mosaic plants were delayed somewhat longer than usual, typical patterns would not develop and the plant would appear healthy. Delayed invasion occurs in the mosaic-resistant Ambalema variety (4), and in Turkish and Burley tobacco with certain strains of the tobacco mosaic virus. The trend of the tobacco virus studies is toward the recognition of numerous strains of relatively few kinds of viruses, several strains frequently originating from one supposedly stable strain. The aucuba mosaic used by Kunkel (3) in his studies may therefore be considered one of the numerous strains of the tobacco mosaic

virus known to exist, as it seems to possess no qualities not found in American strains of tobacco mosaic. If a tobacco plant already affected by one strain of the tobacco mosaic virus fails to develop symptoms when inoculated with another strain, it can hardly be considered as evidence of an acquired immunity, but may simply be proof that cells already affected by this virus cannot be affected by more of the same kind of virus, even tho it is of another strain.[3] The same seems to be true of protection afforded a tobacco plant by a mild strain of the healthy-potato virus (mottle of potato of James Johnson) against a more injurious strain (ringspot of potato of J. J.) (6). In each of these instances the plant may be considered to already have a virulent case of the disease, and immunity would not seem to be concerned. Zinsser (8) has stated that "There is a likelihood of persistence of the viruses in the animal body as long as immunity persists." This suggests that the principles concerned in animal immunity are not well understood. Therefore the conclusion that the principles concerned in the type of protection under discussion in plants are the same as those concerned in immunization by vaccination (3) seems premature. It appears better, for the time being, to speak of protection afforded by one strain of virus against a more injurious strain, bearing in mind the practical application, rather than to speak of an acquired immunity in the sense in which this term is used in animal and human pathology.

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[3] In a paper published by Price since the present paper was written (W. C. Price. Acquired immunity in zinnia. Phytopath. 25, 776-789, 1935) the technic used by Kunkel in his immunity studies was used and with the same result. When plants were "thoroughly invaded" by a virus, reinfection with a necrotic-spotting strain of the same kind of virus failed to give *visible* evidence of infection on the inoculated leaves. If a non-necrotic spotting virus had been used and the plants kept for a sufficiently long time, it would not have been surprising if an occasional plant developed disease from the second virus. The conclusion (page 786) that "zinnia *plants* (italics added) that are infected with tobacco—or aucuba—mosaic virus become immune from infection with virus of tobacco mosaic strain 302-A" cannot be accepted. It is groups of thoroly invaded cells, to which the further addition of the same kind of virus fails to give a reaction, that appear to have become immune and not the plant as a whole. But it is obvious that invaded cells are already diseased and that immunity is therefore not concerned. In paper No. 2 of this bulletin it will be shown that protection is afforded only to invaded cells and not to the partially invaded growing point of a plant which has been affected with mosaic long enough to be producing mosaic patterns in new leaves. It would seem that Price simply presents further evidence that the grouping of tobacco viruses made by Valleau and Johnson (Phytopath. 18, 132-133, 1928) and later amplified by Johnson (Ky. Bul. 306, 1930) is correct.

1. Chester, Kenneth S. The problem of acquired physiological immunity in plants. Quarterly Rev. Biol. 8: 129–154; 276–324. 1933.

2. Henderson, R. G. Transmission of tobacco ringspot by seed of petunia. Phytopath. 21: 225–229. 1931.

3. Kunkel, L. O. Studies on acquired immunity with tobacco and aucuba mosaics. Phytopath. 24: 437–466. 1934.

4. Nolla, J. A. B., and Roque, Arturo. A variety of tobacco resistant to ordinary tobacco mosaic. Journ. Dept. Agric. Puerto Rico 17: 301–303. 1933.

5. Price, W. C. Acquired immunity to ringspot in Nicotiana. Contrib. Boyce Thompson Inst. 4: 359–403. 1932.

6. Salaman, R. N. Protective inoculation against a plant virus. Nature 131: 468. 1933.

7. Valleau, W. D. Seed transmission and sterility studies of two strains of tobacco ringspot. Kentucky Agr. Expt. Sta. Bul. 327. 1932.

8. Zinsser, Hans. On postulates of proof in problems of the bacterial life cycle. Science 75: No. 1940, 256–258. 1932.

2. Susceptibility of Tobacco Plants Visibly Affected with Mild Tobacco Mosaic to Other Strains of the Virus

E. M. JOHNSON and W. D. VALLEAU

In 1926 the writers tested in a limited way the influence that the presence of one virus in a tobacco plant had on development of another virus inoculated into the affected plant. A Turkish tobacco plant inoculated with a mild green mosaic and, 16 days later, with a distorting green mosaic seemed to have only mild mosaic at the end of 28 days. Another Turkish tobacco plant inoculated from it developed symptoms of the severe strain. A White Burley plant, naturally affected with ringspot in the field, was transferred to the greenhouse. The new leaves were without the ring symptoms. The plant was cut back and when the suckers had several leaves they were inoculated with the virus of a mild green mosaic. Ringspot developed in all the suckers, but there were no symptoms of the mild mosaic. A tobacco plant inoculated from the suckers developed mild mosaic in 7 days and ringspot 10 days after inoculation. A Turkish plant inoculated with a mild green mosaic and, 10 days later, with the virus of ringspot, developed necrotic rings on rubbed leaves 6 days after inoculation with the ringspot virus. A Turkish tobacco plant affected with ringspot was inoculated with etch 24 days after the appearance of ringspot symptoms. Symptoms of etch developed in 8 days. A tobacco plant inoculated with the virus of a mild green mosaic and that of ringspot at the same time, devloped symptoms of the former in 7 days and of the latter 16 days after inoculation. These limited tests, together with field observations of numerous mixed infections seemed to prove that while one virus might delay the expression of symptoms of another like or unlike virus, it did not prevent two viruses even of the same kind from multiplying in a plant. There was no evidence of a protective effect except that tissue already invaded by a given virus did not appear to be a good

medium for the multiplication of another, so no further work was done at that time.

The presence of mixed infections in tobacco plants in the field indicates that the presence of one virus affords little protection against infection by another. Ringspot sometimes occurs in the same plant with either tobacco mosaic, cucumber mosaic, etch or veinbanding. Veinbanding often accompanies tobacco and cucumber mosaics. Mixed infections of two or more strains of green tobacco mosaic would be difficult of visual detection, but yellow mosaic is known to occur naturally in the same tobacco plant with a green mosaic.

Papers by Holmes (1), Kunkel (2), Price (3), and others suggest that the presence of certain viruses in a plant may prevent or delay infection from other viruses. Kunkel found that plants of *Nicotiana sylvestris* affected by the virus of a tobacco mosaic or by Aucuba mosaic variants obtained from Aucuba mosaic affected plants held at high temperature, failed to develop necrotic spots, except in the younger leaves, when inoculated with the original Aucuba mosaic virus. Heavy inoculation with tobacco mosaic virus or the virus of an Aucuba mosaic variant was necessary to prevent the development of necrotic spots by Aucuba mosaic. The protected areas were closely confined to the parts inoculated. Price (3) confirmed earlier studies by Wingard (4) in showing that tobacco plants inoculated with ringspot virus soon ceased to form rings and that the new patternless leaves failed to develop rings when again inoculated with the ringspot virus. Apparently "recovered" ringspot tobacco plants seemed less susceptible to infection with tobacco mosaic virus. Holmes (1) isolated a masked strain of tobacco mosaic that infected, increased in, and spread in tobacco plants without causing appreciable mottling, distortion, or stunting. Tobacco plants inoculated with this masked strain and then with a distorting strain of tobacco mosaic, at intervals of 1, 2, and 3 weeks, usually, showed symptoms of the distorting strain later than entirely unprotected plants inoculated with the distorting strain only. The symptoms of the distorting strains were less well defined in the protected plants for a time but the

latter gradually developed symptoms characteristic of the distorting strain, indistinguishable from the symptoms in plants affected with the distorting strain alone. Holmes (1) suggested that the protection afforded by a mild type, tho incomplete, might be of practical value in the field in protecting plants from viruses which cause greater injury. Tomato plants inoculated with the masked strain and set in the field gave better yields of fruit than similar plants inoculated with a distorting strain or uninoculated plants exposed to chance infection.

The tests to be reported were conducted with the object of determining whether protection would be afforded tobacco plants by mild strains of tobacco mosaic, against more severe strains, if the latter virus were introduced after the former had become established in the growing-point leaves. For the inoculations, infected green tissue, crushed in a small amount of water, was rubbed on leaves with sterile cloth swabs.

EXPERIMENTAL WORK

Test 1. (Table 1). Eight uniform Turkish tobacco plants, having 6 to 7 leaves, were inoculated on November 12 by rubbing 4 lower leaves with an extract of a mild green tobacco mosaic. This mild mosaic, designated *a* for convenience, was obtained from a White Burley plant in the field. The leaves were dried, and kept 33 months before being used in these tests. Mottling caused by this virus in tobacco was never prominent and at times was difficult to detect. Affected plants were never distorted and there was little or no stunting. Inoculations after 11 days from unrubbed tip leaves of these 8 plants to *Nicotiana glutinosa* resulted in necrosis in the latter, showing that the mosaic virus had invaded the newer leaves. Fourteen days after inoculation, 4 of the plants were inoculated with the virus of a white tobacco mosaic, and 4 with that of a yellow. At the same time 4 healthy Turkish tobacco plants, comparable in size to the 8, were inoculated, 2 with the white and 2 with the yellow mosaic. Chlorotic spots developed on rubbed leaves and early symptoms of white and yellow mosaic were visible in the unrubbed leaves of these four plants 7 days after inoculation. All the

Table 1. Relative rate of development of white and yellow mosaic in plants previously inoculated with mild green mosaic *a*, and in previously uninoculated plants.

Kind of Mosaic	Green and White	Green and Yellow	White Only	Yellow Only
Number plants inoculated	4	4	2	2
Days from November 26 to appearance of symptoms of the second mosaic on:				
Rubbed leaves	10	10	7	7
Unrubbed leaves	14	14	7	7

8 plants inoculated with mild mosaic *a* and later with white or yellow mosaic, developed local chlorotic spots in 10 days, on leaves rubbed with the latter viruses. Symptoms of white and yellow mosaic were visible in 14 days, on the unrubbed leaves of these 8 plants. The newer leaves of the plants inoculated only with white or yellow mosaic had prominent symptoms in 14 days, whereas the plants affected with mild mosaic prior to inoculation with white or yellow mosaic developed prominent symptoms of the latter in the newer leaves 24 days after inoculation. In this test the presence of a mild green tobacco mosaic in Turkish tobacco merely delayed development of symptoms of white or yellow tobacco mosaic but did not prevent either local or systemic infection (Figure 4).

Test 2. It was believed that if certain areas of inoculated leaves were incompletely invaded by a virus another virus of the same kind entering these areas later would have an opportunity to multiply and from there move into rapidly-growing tissue where invasion by a preceding virus would probably be incomplete. Test 2 (Table 2) was planned to test this hypothesis. Mild mosaic *a* and two other mild mosaics, designated *b* and *c*, both causing more prominent mottling than *a*, were used as the initial invaders. White mosaic was again used as the secondary inoculum. On December 3, 24 uniform Turkish tobacco plants having 6 or 7 leaves were divided into three groups of 8. Each plant was inoculated on 4 lower leaves with one of the

mild green mosaics, *a*, *b*, or *c*. Each lot of 8 plants was again divided into three groups and inoculated on December 18, when symptoms were clearly visible in the upper leaves from the previous inoculation, with the virus of white mosaic, 1 on leaves

Table 2. Inoculation of tobacco plants with mild green mosaic *a*, *b* or *c* December 3, and with white mosaic December 18.

White mosaic was inoculated on.....	Inoculated leaves.	All leaves.	Uninoculated leaves.
Number plants in each group	4	2	2
Days to appearance of white mosaic in new leaves:			
Strain *a*	No infection	10	10–15
Strain *b*	20*	15	15
Strain *c*	60*	18	15
Control, inoculated only with white mosaic, December 18	.....	10	.....

* In one plant only.

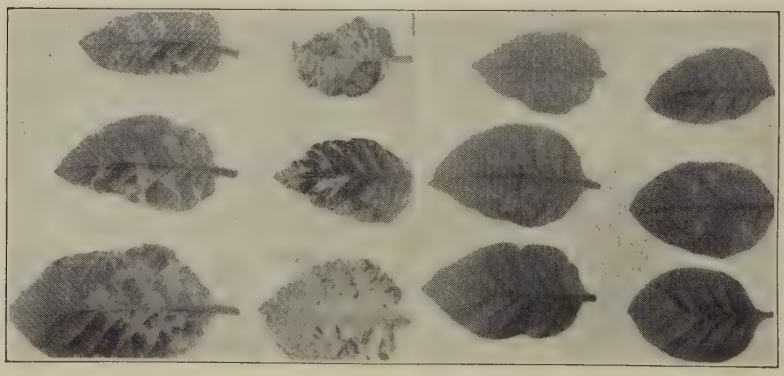

Figure 4. Leaves of Turkish tobacco affected with tobacco mosaic. Photographed Dec. 20, 1934.

Left row. From a plant the lower leaves of which were inoculated on November 12 with a mild green mosaic, and on November 26 with white mosaic.

Second row. From a plant the lower leaves of which were inoculated on November 26 with only white mosaic.

Third row. From a plant the lower leaves of which were inoculated on November 12 with a mild green mosaic and on November 26 with yellow mosaic.

Right row. From a plant the lower leaves of which were inoculated on November 26 with only yellow mosaic.

previously inoculated with mild mosaic, 2 on all the leaves including previously inoculated and uninoculated ones, and 3 on uninoculated leaves only.

None of the four plants inoculated with white mosaic on leaves previously rubbed with mild mosaic *a*, developed symptoms of white mosaic. One of the 4 plants, inoculated with mild mosaic *b* and then with white mosaic on the same leaves, developed white mosaic in the tip leaves in 20 days. White mosaic developed in the tip leaves of one plant in the series inoculated first with mild mosaic *c* and then with white mosaic on the same leaves, 60 days after the second inoculation. All the plants inoculated with mild mosaics *a*, *b*, and *c* and then with white mosaic on all leaves or only on uninoculated leaves situated just above inoculated ones, developed white mosaic in 10 to 18 days after inoculation with the virus of white mosaic. Two healthy Turkish tobacco plants inoculated with the virus of white tobacco mosaic at the same time developed symptoms of white mosaic in the new leaves 10 days after inoculation. These tests prove that if there is tissue incompletely occupied or uninvaded by a virus, a virus of the same kind entering later can multiply and eventually move into the rapidly-expanding incompletely-invaded tissue of the growing point and there express its characteristic symptoms.

Test 3. (Table 3). In a third test, 18 Turkish plants having 6 to 7 leaves, were divided into three groups. One lower leaf of each plant in a group was inoculated with a mild mosaic virus, *a*, *b*, or *c*. Seven days later, after the mild mosaic virus had entered the growing-point leaves, each plant was inoculated, on a single leaf, with the virus of white mosaic. Inocculations were made either on the 1st, 2nd, 3rd, 4th, 5th or 9th leaf above the one inoculated with the virus of mild mosaic. As indicated in Table 3, 10 of the 18 leaves showed mottling of mild mosaic. Seven of these when inoculated with white mosaic developed yellow to almost white spots either at their tips or margins (Figure 5) in 9 to 13 days. These areas being older were probably less completely invaded by the mild mosaic virus. Of the 6 plants inoculated with mild mosaic *a* and then with white

mosaic, all but one developed white mosaic in the new leaves in 12 to 19 days. Only one plant in the series inoculated with mild mosaic *b* developed white mosaic. In Test 2 mild mosaic *b* seemed not to afford any greater protection than *a* or *c*. In this series *b* produced much more striking mottling than either *a* or *c*. Three plants in the series inoculated with mild mosaic *c* prior to inoculation with white mosaic developed symptoms of the latter virus in 16 to 19 days after inoculation. In all the mild-

Figure 5. Turkish tobacco plants systemically infected with mild green mosaic, showing local chlorotic spots (see arrows) on leaves inoculated later with white mosaic. The plants with 7 leaves were inoculated on March 9 with mild mosaic on one of the lower leaves. The leaves indicated by arrows were inoculated March 16 with white mosaic after growing-point symptoms of mild mosaic had developed. Symptoms of white mosaic were visible in the growing-point leaves, 7 to 9 inches above the white mosaic-inoculated leaf, 19 days later. Photographed April 12, 1935.

mosaic plants which subsequently developed symptoms of white mosaic in the growing point, the symptoms of the latter were less striking than in plants affected with only white mosaic. Mild mosaic-affected plants did not develop prominent symptoms of white mosaic until after 25 days, whereas plants inoculated only with white mosaic virus usually developed prominent symptoms in 10 to 15 days. The inoculations in Test 3 again indicate that mild tobacco mosaics delay the development of symptoms of white mosaic but do not prevent systemic infection if there

TABLE 3. Development of white mosaic in plants previously inoculated with a mild green mosaic.

Plant No.	One leaf inoculated on March 9 with mild mosaic	Inoculated with white mosaic on March 16 on leaf*	Days to appearance of white mosaic in new leaves
1	a	2	12
2	a	3	19
3	a	4	16
4	a	5 (mottled)	16
5	a	6 (mottled)	16
6	a	9 (mottled)	....
7	b	2	....
8	b	3	16
9	b	4 (mottled)	....
10	b	5 (mottled)	....
11	b	6 (mottled)	....
12	b	9 (mottled)	....
13	c	2	....
14	c	3	19
15	c	4	19
16	c	5 (mottled)	....
17	c	6 (mottled)	16
18	c	9 (mottled)	....

* The leaf inoculated with mild mosaic designated number 1.

are areas in which the latter can multiply. Also the results indicate that systemic infection is less likely to occur with a second virus if inoculations are made on younger leaves where invasion is likely to be more complete.

DISCUSSION

The tests seem to prove that if a tobacco leaf inoculated with a second strain of a virus is unoccupied or only partly occupied by the first strain, so that the second may multiply, the second virus will eventually be transported to the growing point where it will have an opportunity for multiplication equal to that of the first strain. The growing point of a tobacco plant appears to be entirely unprotected by one strain of a tobacco mosaic virus against another strain if the latter has sufficient uninvaded tissue in which to multiply and from which it may be transported to the growing point. Protection is afforded individual cells or groups of cells, perhaps, but the plant as a whole does not develop immunity.

SUMMARY

Turkish tobacco plants having 6 to 7 leaves were inoculated with various natural strains of mild green tobacco mosaic and, 7 to 15 days later, when infection was systemic, with the virus of white or yellow tobacco mosaic. When mild mosaic plants were inoculated with either white or yellow tobacco mosaic on leaves not previously inoculated, they developed symptoms of white or yellow tobacco mosaic in the tip leaves in 10 to 19 days. Similar plants inoculated with either white or yellow mosaic only, showed symptoms in the tip leaves in 7 to 10 days. When inoculations were made with white mosaic on leaves previously rubbed with mild mosaic, symptoms of white mosaic were visible in the new leaves of some of the plants in 20 to 60 days.

Symptoms of white or yellow mosaic were slightly less conspicuous and appeared later in plants affected with mild mosaic than in plants affected with only white or yellow mosaic.

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3. Price, W. C. Acquired immunity to ringspot in Nicotiana. Cont. Boyce Thompson Inst. 4: 359–403. 1932.

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3. Localization and Resistance to Tobacco Mosaic, in Nicotiana

W. D. VALLEAU

Recent studies on tobacco mosaic suggest that the disease might be controlled if varieties of tobacco could be found in which the virus is localized in necrotic lesions at the points of inoculation. Kunkel (6) found that several varieties of *Nicotiana tabacum,* when inoculated with the English tomato aucuba mosaic, developed local necrotic lesions, but usually failed to become systemically infected. He found that certain other species of Nicotiana, when inoculated with the tobacco-mosaic virus, developed local necrotic lesions in which the virus appeared to be localized. Holmes (4) found that the genetic factor controlling necrotic spotting is a Mendelian dominant in the Nicotiana species studied, and in some other genera of Solanaceæ. He claimed that "a plant that possesses the dominant character is able to restrain tobacco-mosaic virus from producing a systemic infection, although without this factor it would be unable to do so."

Seed was obtained from the Rockefeller Institute, of the various Nicotiana species which had shown the phenomenon of necrotic spotting. The seed was obtained for the purpose of hybridizing these species with *N. tabacum,* and for a further study of localization. Also a study was made of *Nicotiana tabacum* hybrids which were being grown in the greenhouse for other purposes, to see if the English aucuba mosaic of tomato (a strain of the tobacco mosaic virus) would cause necrotic spotting and not become systemic. A third study was made to discover whether any tobacco-mosaic viruses collected in Kentucky likewise would cause necrotic spotting and not become systemic in varieties of tobacco carrying the necrotic factor. Finally, a study was made of about one hundred varieties and hybrids of *N. tabacum,* to determine their reaction to white mosaic, a nec-

rotic-spotting virus. Results of these studies do not offer much hope of control of tobacco mosaic thru localization of the virus in necrotic lesions; for tobacco varieties exhibit the phenomenon of local necrotic spotting to but a few strains of the virus, and localization does not always accompany necrotic spotting in all varieties of *N. tabacum* or in all species of Nicotiana in which the phenomenon of local necrotic spotting occurs. The hypothesis of localization is inadequate to explain what occurs in necrotic-spotting plants following inoculation with a necrotic-spotting virus.

Ten years ago, Fernow (2) described necrotic local spotting of potato leaves inoculated with the tobacco-mosaic virus, and this type of spotting has been reported several times since in other genera. For example, E. M. Johnson (5) stated that on Jimson "all tobacco mosaics, regardless of type, produce the same symptom. In three to eleven days, inoculated plants develop circular spots 1/16 to ¼ inch in diameter on rubbed or pricked leaves. A week later, necrotic streaks develop on the midribs and petioles of these leaves and usually spread down the stems. Infection seems almost entirely local, as unrubbed leaves have shown necrosis but once." He stated further that in *Nicotiana rustica* "six tobacco mosaics produced the same symptoms on this species. Small, brown, circular, water-soaked spots develop on rubbed leaves six to thirteen days after inoculation. A few days later, similar spots develop on unrubbed leaves. These spots increase in size rapidly and kill the affected leaves." Systemic infection followed in all inoculated plants. Fernow (2) and Holmes (3) reported similar results with *N. rustica*. Valleau and Johnson (9) reported necrotic spotting on rubbed leaves of potatoes, accompanied by necrotic stem streaking, and found that the virus did not become systemic. While localization was recognized in some instances in these studies, the interpretation that necrotic spotting results in localization and is the reason for failure of systemic infection did not appear to be in accord with observed facts and was given no further consideration by the writer. In the present paper the results of inoculation experiments on tobacco varieties and

hybrids and on *Nicotiana* species in which localization is presumed to occur are reported, and a theory is presented as to what takes place in socalled localization of the virus in these forms.

STRAINS OF TOBACCO MOSAIC WHICH CAUSE NECROTIC SPOTTING IN N. TABACUM

Fifty-nine strains of mosaic[1] collected during the past 10 years and preserved by drying were tested on necrotic-spotting tobacco varieties. Seven strains caused necrotic spots; five caused a few small necrotic spots or only necrotic rings; forty-seven caused no local symptoms whatever on these varieties. The necrotic-spotting strains originated from pepper (two strains), ground cherry (two strains), tomato (two strains—1, a severe streak in the field; and 2, a streak collected by Dr. A. L. Pierstorff in a greenhouse in Ohio), tobacco [one strain, ring mosaic (5)]. Four strains which produced slight necrosis in the form of faint necrotic rings were all obtained from Kentucky White Burley No. 5 tobacco plants in the field, which had unusual cases of yellow tobacco mosaic. A fifth strain was a yellow tobacco mosaic collected by Dr. S. G. Lehman in North Carolina. The remaining viruses which produced no necrosis were nearly all collected from tobacco. It appears likely, from these results, that the necrotic-spot-producing viruses, while present in native weeds, rarely become established in White Burley tobacco growing in the field, perhaps because most of the commonly-grown varieties carry the necrosis factor.

The symptoms produced on Turkish tobacco by the necrotic-spotting mosaic strains ranged from extremely mild mottling with no distortion to severe distortion, and from green to white. It is probable that no sharp line can be drawn between the necrosis-producing strains and others. On the same leaf, one strain may produce large necrotic spots, while another may produce minute ones, and a third strain may produce none whatever

[1] In this paper the term mosaic refers to the disease caused by one of the strains of the tobacco mosaic virus. This virus is characterized by its ability to survive for years in a dry state and by thermal inactivation between 80° and 90° C. for 10 minutes. The common field mosaic of tobacco may be any one of several strains of this virus and not a single one as is commonly assumed.

(Figure 6). A strain which produces large necrotic spots on a full-grown leaf of *N. tabacum* may produce only small, faint necrotic rings on a younger leaf of the same plant (Figue 7), whereas another strain may produce only faint rings on the older leaves. On other species, as *N. glutinosa* or *N. acuminata,* all tobacco mosaic strains tested produced necrotic spots, but the

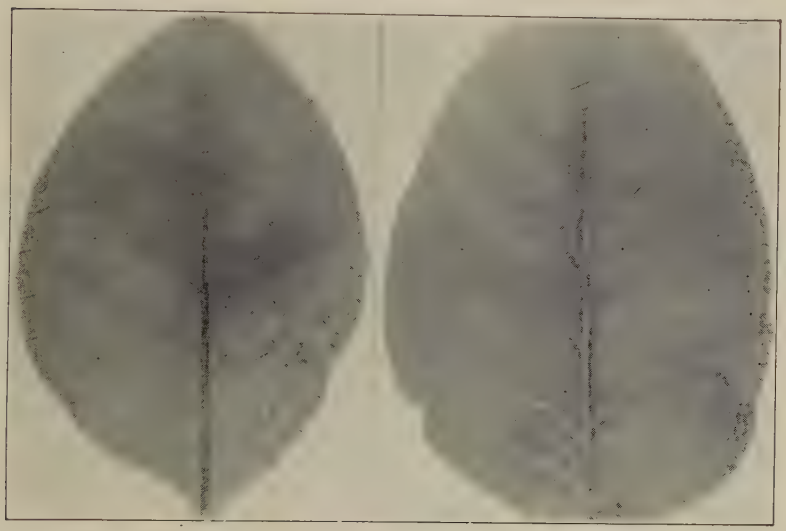

Figure 6. Leaves of F_1 Judy's Pride White Burley (necrotic spotting) x Turkish (non-necrotic spotting) each inoculated with 4 different strains of tobacco mosaic. Left: Upper left ¼, a yellow mosaic, no spotting; upper right ¼, another yellow mosaic, necrotic spots numerous but minute; lower left ¼, another yellow mosaic, necrotic spots few and minute; lower right white mosaic, necrotic areas large. Right: Upper left, green distorting mosaic, necrotic spots numerous, small; upper right, green, slightly distorting mosaic, no necrotic spots; lower left, yellow speckled mosaic, no necrotic spots; lower right, distorting green mosaic, necrotic spots numerous, small.

spots produced by different strains were often different. On *N. rustica,* local infection may vary from sharply limited necrotic spots to necrotic rings surrounding chlorotic tissue, according to the virus strain used and the age of the leaf inoculated.

LOSS OF NECROTIC FACTOR OF WHITE MOSAIC VIRUS

Several instances of loss of ability of a virus to cause necrotic spotting in tobacco at the point of inoculation have been observed

by the writer. Two were of loss from the necrotic-spotting white mosaic virus (5). One non-necrotic strain was obtained in a transfer from a white-mosaic plant to another Turkish plant. Evidently the change had occurred in the former plant where a green variant gradually occupied the growing point, which at first was a typical white mosaic. An Ambalema plant (in which

Figure 7. A necrotic-spotting F_1 hybrid of Baur low-nicotine (necrotic spotting) x Ky. White Burley 14 (non-necrotic spotting) inoculated with a yellow mosaic (non-necrotic spotting) left, and aucuba mosaic (necrotic spotting) right. The leaves were not fully grown when inoculated. The spots on the left leaf were yellow chlorotic areas. Those on the right were slightly chlorotic spots surrounded by a necrotic ring. This type of spot is frequently developed by the necrotic-spotting viruses on immature leaves of necrotic-spotting plants. Plants from which these leaves were removed both developed systemic infection; one (left) a non-necrotic, yellow mosaic, and the other (right) streak.

white mosaic completely localizes), inoculated with this virus dried for thirty-one months in tobacco, was gradually invaded. Chlorotic and necrotic ring patterns appeared as the leaves attained nearly full size (Figure 8). This virus, transferred to Turkish tobacco, produced a green mosaic and failed to pro-

duce necrotic spots on Ambalema and other necrotic-spotting varieties. The other strain was derived from a strain of the same white mosaic, which was believed to be pure. An inoculation made to a young Ambalema plant caused necrotic spots on inoculated leaves. A chlorotic ring pattern developed on the tip of one uninoculated leaf, and from this a non-spotting green

Figure 8. A white mosaic variant in Ambalema tobacco. Soon after the appearance of necrotic patterns (left) the leaf gradually dies back from the tip (right). It seems evident that the necrotic factor has not been lost completely from this strain of the virus.

strain was obtained. Failure of this virus to become systemic in Ambalema differentiated it from the first isolant; however, the ability of both virus strains to cause identical chlorotic and necrotic patterns in inoculated leaves of Ambalema, rather than necrotic local spots, seems to indicate a common origin, as

this particular type of chlorosis and necrosis was caused by no other virus strain studied in Ambalema. Instances of the loss of the necrosis factor from other strains of mosaic virus in Ambalema have been noted by the writer, but the evidence for purity of the original virus was not so good as in the case of white mosaic. Kunkel (6) reported two instances of the loss of ability of the aucuba mosaic virus to produce necrosis in *N. sylvestris* when a plant of this species was inoculated with aucuba mosaic and then held for three days at a temperature of 35 degrees, C. Having lost the ability to cause necrosis, it became systemic in *N. sylvestris* at a lower temperature. The rather frequent changes in viruses in Ambalema seem to correspond to those which occur in inoculated plants carried at 35° C., which are sometimes considered as an attenuation of the virus.

VARIETIES OF NICOTIANA TABACUM WHICH DEVELOP NECROTIC SPOTS

The reaction of nearly one hundred *Nicotiana tabacum* hybrids and varieties to white and aucuba mosaic viruses was studied. The plants used were grown in the greenhouse or in out-of-door beds and later transferred to the greenhouse. It was found, after inoculation, that the varieties could be separated into two distinct groups; those in which necrotic spots developed, and those in which large chlorotic yellow to orange local spots developed (Figures 6 and 7). In the necrotic-spotting group were fourteen commonly grown Kentucky varieties of tobacco and sixty-one *N. tabacum* hybrids. Only four varieties, two of which are grown in Kentucky, and fifty-four hybrids developed chlorotic spots. Turkish was one of the varieties reacting in this way. The chlorotic-spotting varieties invariably developed mosaic typical of the virus used.

The necrotic-spotting varieties and hybrids could further be classified into: 1, those in which the necrotic spots developed rapidly and the virus finally became localized within the inoculated leaf, or which produced a severe necrotic disease of the streak type accompanied by stunting (Figure 9); 2, those in which local necrotic spotting occurred and was followed by sys-

temic infection of a mild streak type and by slight stunting; 3, F_1 hybrids of Ambalema and chlorotic-spotting varieties in which necrotic spotting developed more slowly on old inoculated leaves, and chlorotic to stipple-necrotic concentric rings developed on younger inoculated leaves. Systemic infection was common in these hybrids and was usually accompanied by some

Figure 9. Illustrating sensitivity of necrotic-spotting plants to necrotic-spotting viruses. F_1 hybrids of Ky. 115 (necrotic spotting) x Ambalema (necrotic spotting) inoculated with white mosaic (left) and aucuba mosaic (right). This hybrid is more sensitive to the necrotic-spotting viruse than F_1 hybrids of non-necrotic-spotting varieties with Ambalema, probably because the former is homozygous for the more necrotic-spotting factor. This hybrid usually did not develop systemic infection but in this series of 21 plants each was inoculated on ½ of each leaf, with one of 3 necrotic-spotting viruses. Petiole necrosis developed on the inoculated leaves, necrotic streaks in the stem, and vein necrosis in the young leaves. The plants made no further growth.

necrosis (Figures 2 and 10). The type of mosaic produced was not typical white or aucuba mosaic, but was of the ring-mosaic type. The development of ring patterns suggests that the hybrids were more resistant than the susceptible parent. Sys-

temic infection was accompanied by slight midvein necrosis of inoculated leaves and slight stem streak.

FIELD TESTS OF WHITE MOSAIC ON NECROTIC-SPOTTING VARIETIES

Field infection tests with white mosaic and three non-necrotic-spotting strains of tobacco-mosaic virus were made on fifty-

Figure 10. The effect of white mosaic on an F_1 hybrid of White Burley 5 (non-necrotic spotting) x Ambalema (necrotic spotting), left; and Ambalema, right. Both plants were inoculated on 2 leaves. Necrotic spots are evident on the lower, older leaf of the hybrid (left) and stipple necrosis on the younger inoculated leaf (upper). Systemic infection with slight stem streaking usually occurs in plants of this genetic constitution. Compare with Figure 9. Necrotic spots and rings developed on inoculated leaves of Ambalema but systemic infection did not develop.

six hybrids and varieties of Burley and dark tobacco, to determine the extent to which the necrotic-spotting factor might protect field-grown plants from white mosaic, and to determine the type of mosaic produced on necrotic-spotting and non-spotting

varieties. Nearly all the White Burley hybrids produced in breeding for black-root-rot resistance were of the chlorotic-spotting type, while all the dark tobacco hybrids were of the necrotic-spotting type.

Twenty-six varieties and hybrids (not including Ambalema hybrids) of which only three were White Burley, were of the necrotic-spotting type. Five plants of each variety were inoculated with each of the four virus strains. The results, as far as systemic infection is concerned, are given in Table 1.

Table 1. Systemic infection in twenty-six varieties of tobacco inoculated with four different tobacco-mosaic viruses. Five plants of each variety were inoculated with each virus.

Class	Number of varieties in each class, after inoculation with			
	White mosaic	Severe mosaic	Mosaic burn	Ring mosaic*
No plant infected	3	0	0	0
1 plant infected	1	0	0	2
2 plants infected	5	0	0	4
3 plants infected	6	0	0	7
4 plants infected	6	0	1	5
5 plants infected	5	26	25	8
Total plants with mosaic	78	130	129	91
Percent infected	60	100	99	70

* This ring mosaic differs from the one described by E. M. Johnson (5). It is ordinarily non-distorting and non-mottling, and appears to invade the growing-point leaves rather slowly.

Necrotic-spotting plants were considerably protected against white mosaic (60 percent infection), but not against severe and mosaic-burn viruses, which caused 100 and 99 percent infection, respectively. Necrotic spots appeared on the small area of the leaf inoculated with white mosaic, but in most instances necrosis of the large veins in the inoculated area, and of the midvein developed, so that in many of the protected plants the virus spread for several inches in the inoculated leaf. Delayed systemic infection was frequent in this group. Fifty-

two of fifty-five plants of non-necrotic-spotting varieties of White Burley, inoculated with white mosaic, or 95 percent, developed systemic infection. Ring mosaic, a non-necrotic-spotting virus, caused systemic infection in only 70 percent of the necrotic-spotting varieties, and in 85 percent of non-spotting varieties of White Burley. Failure of the non-spotting ring mosaic to produce infection in 28 percent of the plants suggests that other factors than necrosis may be involved in failure of plants to become systematically infected with this virus, at least.

When systemic infection occurred in necrotic-spotting varieties inoculated with white mosaic, the disease produced was different from that produced in the non-necrotic-spotting varieties. The necrotic-spotting varieties developed typical streak with necrosis of the stem extending well into the pith. In the non-necrotic-spotting varieties, white mosaic developed without necrosis. In these, as in the greenhouse tests, local necrotic spotting may be taken as evidence of a high degree of sensitivity of the plant cells thruout the plant, to the white-mosaic virus.

The mosaic-resistant Ambalema tobacco was inoculated with the same four strains of the mosaic virus. A few plants showed mild symptoms of mosaic, but these were a broader-leafed type of tobacco differing from the mosaic resistant plants which showed no evidence of infection. Five full-grown Ambalema plants in the field were inoculated on all leaves with the white mosaic virus, but ten days later showed no evidence whatever of having been inoculated.

F_1 hybrids between Ambalema and White Burley all bore the necrotic-spotting factor from the Ambalema parent only. In Ambalema x Ky. 115 (dark) and its reciprocal, the F_1 plants received the necrotic spotting factor from Kentucky 115 also. Protection conferred by the single factor from Ambalema appeared to be slight, because in sixty-five plants of fourteen hybrids or reciprocals, fifty-eight, or 90 percent, developed white mosaic. The disease was of the non-distorting type which causes only slightly retarded growth of the plant. Chlorotic ring patterns were the rule. When two necrotic-spotting factors were

present, one from Ambalema and one from Ky. 115, greater protection was conferred (altho the numbers were small), as ten plants of this hybrid and its reciprocal, inoculated with white mosaic, developed only five cases of mild light green mosaic (50 percent protection), much less noticeable than the prominent white mosaic in Ambalema x Burley hybrids.

INHERITANCE OF THE NECROTIC SPOTTING OR SENSITIVITY FACTOR

Holmes (4) showed that the factor controlling necrotic spotting in several species of *Nicotiana* and in other genera is a Mendelian dominant. In the present studies, several hybrids between varieties of *N. tabacum* have shown that the sensitivity factor is a Mendelian dominant in *N. tabacum* also. For example, F_1 hybrids between Judy's Pride (a necrotic-spotting variety) and Turkish (a non-spotting variety) developed necrotic spots when inoculated with white or aucuba mosaic viruses. Twenty-two F_2 plants segregated into sixteen with necrotic spots and six with chlorotic spots. The necrotic-spotting plants developed the necrotic or streak type of systemic infection, and the others the non-necrotic type of mosaic. F_1 hybrids of Ambalema (necrotic-spotting) with chlorotic-spotting varieties were all necrotic-spotting, altho sometimes only very slight necrosis developed. F_2 hybrids segregated into approximately ¼ chlorotic spotting with non-necrotic systemic infection; ½ necrotic ring spotting with somewhat necrotic systemic infection, and ¼ necrotic spotting without systemic infection or with very severe streak. Presumably the latter two groups are heterozygous and homozygous for the necrotic spotting factors respectively. If this proves to be the case, then the necrotic spotting factor of Ambalema is only partially dominant. (Compare Figures 2 and 9.)

Hybrids of White Burley No. 5 (chlorotic-spotting) x *N. glutinosa* (necrotic-spotting) gave only necrotic-spotting plants in the F_1, confirming the results of Holmes (4). Segregation of other hybrids of *N. tabacum* varieties studied may be similarly interpreted. F_1 hybrids between a chlorotic-spotting variety

and a plant of Kelley (usually necrotic spotting) selected at random from a row of this variety, were all chlorotic spotting The Kelley variety is therefore heterozygous for the necrotic-spotting factor and its recessive allelomorph. F_1 hybrids in which both parents were chlorotic spotting (recessive) gave only chlorotic-spotting plants in the progeny. While the tests conducted on hybrids were not made for the purpose of studying inheritance of necrotic spotting, yet they confirm the conclusion of Holmes, that necrotic spotting is dominant over chlorotic spotting in *N. tabacum* as in other species of *Nicotiana* and other genera of Solonaceae. Sensitivity as indicated by degrees of necrosis when systemic infection develops, is only partially dominant. The necrotic spotting and sensitivity factors are identical.

REACTION OF THE MOSAIC-RESISTANT AMBALEMA[2] TO NECROTIC AND NON-NECROTIC VIRUSES

In Table 2 are given the condensed results of inoculation of ninety-two mosaic-resistant Ambalema plants with thirty-eight mosaic strains, most of which were found occurring naturally, while a few were isolants.

In Ambalema even in young, very tender plants, necrotic-spotting mosaic strains rarely, if ever, become systemic (Figure 10). Where systemic infection occurred, the virus which became systemic differed in symptom expression in Turkish tobacco from the original virus. Field-grown plants of Ambalema, inoculated with the white-mosaic virus, failed to show any local symptoms whatever, even when all leaves were inoculated, and failed to develop systemic infection.

A plant of Ambalema, on which a cion of Turkish tobacco was grafted and later inoculated with white mosaic, produced a lateral branch several weeks later. There developed on the young leaves, a short distance below the growing point of this rapidly growing lateral, numerous chlorotic spots which gradually changed to stipple-necrotic rings. Inoculations from these

[2] Seed of Ambalema (7) was obtained from Dr. Nolla to whom the writer is grateful. A more complete account of Dr. Nolla's studies is given in Jour. of Agr. Univ. of Puerto Rico 19:29-49, 1935.

spots to susceptible plants showed that they contained the white-mosaic virus. Virus particles produced in the Turkish tobacco cion were carried into the several leaves of Ambalema, where they caused the same reaction as tho the leaves had been inoculated. This instance seems to prove that virus particles are carried long distances from the point of multiplication of the virus even in a mosaic resistant variety.

Table 2. Type of infection produced on Ambalema following inoculation with tobacco-mosaic viruses which cause necrotic spotting, necrotic rings or no necrosis on the inoculated leaf.

Local symptoms	Type of infection	Number of plants
Necrotic spotting	Not systemic	21
Necrotic spotting	Systemic	3*
No necrosis	Systemic	53
No necrosis	Not systemic	9**
Slight necrosis (rings)	Systemic	4
Slight necrosis (rings)	Not systemic	2

* A different virus re-isolated.
** Four were N-starved plants.

It is shown earlier in this paper that the genetic factor controlling necrotic spotting in Ambalema does not prevent systemic infection in F_1 hybrids between Ambalema and non-necrotic-spotting varieties. F_1 hybrids of Ambalema with necrotic-spotting varieties develop systemic infection less frequently, probably because of the presence of two necrotic-spotting factors.

The non-necrosis-producing viruses usually invade plants of Ambalema slowly. Mosaic symptoms have not been observed in growing-point leaves, altho they sometimes developed gradually in the older leaves, and then usually in the form of cholorotic or necrotic ring patterns (Figure 8). There appears to be a distinct difference in the rate of invasion of the different virus strains into young leaves. Some enter leaves close to the growing point, and others lag well behind the growing point. Most strains of tobacco-mosaic virus fail to produce any visible symptoms in Ambalema, even tho the virus may be present in all but the growing-point leaves.

Resistance to Mosaic. While the primary subject under discussion in this paper is the problem of the sensitivity of tobacco to strains of the tobacco-mosaic virus, it may not be out of place to discuss here resistance of tobacco varieties to the mosaic virus. The variety, Ambelema, appears to be resistant to mosaic for two reasons: 1, because necrosis-producing strains of the virus appear to be completely localized, even in very young plants; and 2, because of slow multiplication and the apparent inability of the mosaic virus to invade young tissue of this variety, as evidenced by failure to demonstrate the virus in transfers to Turkish tobacco or to *N. glutinosa* from growing point leaves of Ambalema plants, inoculated with numerous strains of mosaic. When infected Ambalema plants are forced into growth by pruning back to a point where the leaves are known to contain the virus, the shoots develop without symptoms and are at first virus-free, altho they have been in close proximity to the virus for weeks. This seems further proof that the virus does not readily enter meristematic tissue. The virus soon invades the older leaves of these new shoots.

The new leaves of plants of Turkish tobacco and other varieties systemically infected with certain strains of tobacco mosaic frequently develop to some size without patterns appearing on them. Considerable areas of these leaves are free from virus whether or not the leaf as a whole is free. This was demonstrated by using cork-borer sections from these leaves as inoculum on healthy plants where some of the sections failed to cause mosaic. Either chlorotic or necrotic ring and line patterns appear on leaves following somewhat delayed invasion. Kunkel (6) demonstrated that very young leaves of *N. sylvestris* plants infected with tobacco mosaic were not completely invaded by virus, for when these leaves were inoculated with aucuba mosaic, necrotic spots developed, but not in as great numbers as when healthy leaves were inoculated. This seems to indicate that mottling in tobacco plants is caused by resistance of meristematic tissue to invasion by the virus. Meristematic tissue seems to delay invasion until after the leaf cells are differentiated, or until differentiation of vascular tissues perhaps has opened nat-

ural channels for transportation of the virus. If invasion of the growing point were complete, it is probable that subsequent leaves would be patternless and, in the case of the green mosaics, the plants would appear as symptomless carriers. Thus it is possible that symptomless plants might result either from complete occupation of the growing point or from delayed invasion such as occurs in Ambalema, or by invasion by a virus so mild in its effect on chlorophyl that patterns are not evident. Some of the yellow and white mosaic viruses, because of delayed invasion, cause chlorotic or necrotic ring-spot patterns on Turkish, White Burley, and Ambalema. It appears probable, from the evidence at hand, that there is only a slight difference in resistance between Ambalema and other *N. tabacum* varieties. Resistance in Ambalema is sufficient to delay invasion of all strains of tobacco mosaic until growing-point leaves have attained considerable size.

DELAYED INVASION AND SEED TRANSMISSION OF VIRUSES

In inoculation experiments in which very young leaves of so-called recovered ringspot plants are used as inoculum, infection occurs regularly, which indicates that very young tissue is invaded. Seed transmission of the virus occurs rather frequently. Inoculations in which very young leaves of Turkish tobacco plants infected with certain tobacco-mosaic viruses are used for inoculum do not regularly give infection. This indicates that the growing point is not completely invaded by the virus. Inoculations from growing-point leaves of Ambalema regularly fail to cause infection. The fact that ringspot is sometimes transmitted in the seed and mosaic is not, suggests that meristematic tissue is resistant to the virus of mosaic, but somewhat susceptible to the virus of ringspot.

INFECTION EXPERIMENTS WITH OTHER SPECIES OF NICOTIANA

Nicotiana glutinosa plants inoculated with any of the strains of the tobacco-mosaic virus tested developed necrotic spots at the points of entrance of the virus. The spots continued to enlarge

and, if numerous, the tissue between the spots became involved and the inoculated portion of the leaf died. Individual spots sometimes attained a diameter of 2 cm. The virus usually failed to become systemic, but occasionally necrosis spread down the under side of the midvein, continued down the petiole and into

Figure 11. Sensitivity of *Nicotiana glutinosa* to tobacco mosaic. C. Two leaves inoculated with tobacco mosaic 3 and 7 days previously, illustrating increase in size of the spots. D. Petiole necrosis on leaves of which only the blade was inoculated. B. Stem streak resulting from inoculation of lower leaves. A. Systemic infection showing leafspot on uninoculated leaves. Altho the virus spread into the young leaves, the tissues were so sensitive that death resulted in areas where the virus particles lodged and multiplied.

the stalk, and produced a black streak. When several leaves were inoculated, girdling and death of several inches of stalk was not unusual. At least one strain of the virus was isolated from a yellow mosaic by E. M. Johnson, which he found became systemic in some plants of *N. glutinosa.* This strain caused a severe necrotic disease of uninoculated leaves, then death of the growing point, and finally death of the whole plant (Figure 11). Usually there was good evidence of localization of the tobacco-mosaic virus in *N. glutinosa;* but the gradual coalescence of spots, petiole necrosis, stem streaking, and sometimes systemic infection, proved that localization was not complete (Figure 11).

When plants of *Nicotiana acuminata* in the rosette stage were inoculated with four strains of mosaic which varied from a very mild, nearly patternless type to a distorting type, local necrotic lesions developed on each inoculated leaf. The inoculated leaves gradually died, and the virus spread into the petiole. The growing-point leaves of plants kept for further observation became irregularly chlorotic; the plants grew slowly; and the lower leaves either wilted quickly or died from the base of the petiole outward. When split plants were examined, it was seen that necrosis of the petioles of inoculated leaves had continued into the stem and killed a cross section of it. A single plant of this species just coming into bloom was inoculated on the tip of each leaf, with tobacco mosaic. The leaves on the lower half of the plant died back from the tip to the point of attachment, and a narrow necrotic streak spread down the stem from the base of each petiole. In this species, altho the virus seemed not to become completely systemic, it was not localized about the points of inoculation or in the inoculated leaf, but spreads into the stalk, slowly killing invaded tissue. This species appears to be slightly less sensitive to the tobacco mosaic virus than *N. glutinosa.*

In *Nicotiana sanderae,* necrotic lesions developed at the points of inoculation. The tissues between the spots gradually died, but the remainder of the inoculated leaf remained alive for a long time. Typical mosaic patterns did not immediately develop in the new leaves of these plants; but in an occasional

leaf, chlorotic rings, chlorotic spots, or necrotic spots appeared according to the strain of virus used. A plant inoculated with severe mosaic developed systemic infection, and necrotic spots in uninoculated, invaded leaves. Seven inoculations to *N. glutinosa* were made, with cork-borer sections of spots from the eighth and ninth leaves above the inoculated leaves, as inoculum.

Figure 12. Contact print of a leaf of *N. langsdorffii* inoculated in the rosette stage with mild tobacco mosaic. Following necrotic spotting of the rubbed leaves, the ringspot type of pattern developed in nearly all leaves. The virus concentration in these leaves was very low.

Five inoculations caused necrotic spots on inoculated leaves of *N. glutinosa*, which proved that the virus had become partially systemic in *N. sanderae*. The lower leaves of the *N. sanderae* plant died from no apparent cause, but when the stem

was split, large necrotic pits were found which extended into the pith from the bases of the dead leaves. A second plant inoculated with mild mosaic 1 (5) developed chlorotic spots on uninoculated leaves. Inoculations from spots on the fifth leaf and a higher one above the inoculated leaf caused typical necrotic spots in *N. glutinosa* in each of three instances. The growing points of this *N. sanderae* plant gradually became mottled and some large necrotic spots developed. Three months after inoculation, mild mosaic was transferred from this plant to four Turkish plants. A plant inoculated with a very mild mosaic gave no evidence of systemic infection until about three months after inoculation, when faint oak-leaf patterns developed on rosette leaves. The virus failed to transfer to Turkish tobacco from the patterns. Two other plants inoculated with mild mosaics developed systemic infection. Nine plants were inoculated with individual strains of the mosaic virus. Each virus caused local necrotic spotting, and four caused systemic infection within a few days after the appearance of necrotic spots on inoculated leaves. Ten plants developed necrotic spotting on inoculated leaves, but in each instance the virus failed to become systemic. The plants had been growing in tumblers for a longer period than those in the previous series, and the inoculated leaves were relatively more mature. This species appears to be less sensitive than the two preceding species, as occasionally systemic infection with mottling occurred.

Nicotiana langsdorffii plants inoculated with four strains of the virus developed necrotic spots on inoculated leaves. In two plants, typical mosaic patterns developed in the new leaves, leaving no doubt of systemic infection. In a third plant, necrotic oak-leaf patterns gradually spread along the midveins of uninoculated leaves and continued to spread thruout the growth of the plant (Figure 12). All attempts to transfer a virus from necrotic leaves to either *N. glutinosa* or to Turkish tobacco failed. In a fourth plant inoculated with a very mild strain of tobacco mosaic, chlorotic spots developed in uninoculated leaves. Inoculations with cork-borer sections of chlorotic areas on leaves 1, 3, 5, 8, and 12 above the inoculated leaves, eight inoculations in all,

each produced necrotic local lesions on *N. glutinosa.* This is usually considered proof of the presence of tobacco-mosaic virus. The particular virus used in this instance was from dry tobacco tissue several years old, was passed thru one Turkish plant and then to the plant of *N. langsdorffii* in question. One hundred days after the inoculation, the growing points of this plant were mottled; several lateral shoots had died; and transfers to four Turkish tobacco plants each reproduced the very mild mosaic. Nine plants were inoculated when about five leaves were present in the rosette. A different strain of tobacco mosaic was used on each plant. Necrotic spots developed on each of two inoculated leaves on each plant, and systemic infection of the second and third leaves above the inoculated ones followed in a few days. Most of these plants wilted during the next week and, when split open, were found to have extensive necrotic cavities thruout the pith. Necrotic spotting in *N. langsdorffii* did not necessarily prevent systemic infection. This species appears to be less sensitive than the three preceding species, consequently it is more likely to develop systemic infection.

Nicotiana rustica plants were studied by E. M. Johnson (5), who described local necrotic spotting on inoculated leaves, necrosis and distortion in subsequent leaves, and, frequently, death of the plant in the field. Local necrotic spotting did not cause localization of the virus in the variety used. The writer has repeated this work with a strain of *N. rustica* obtained from the Pennsylvania Experiment Station. Ten plants inoculated with individual strains of tobacco mosaic each developed necrotic spotting on the inoculated half of a lower leaf. Necrotic spotting was followed by systemic infection of the second and younger leaves above the inoculated leaf. Altho necrotic spotting occurred on inoculated leaves, there was no evidence of localization of the virus either at the points of inoculation or in the inoculated leaf.

Other plants, which were older, were each inoculated on the tip of one leaf. None of these plants developed systemic infection. The inoculated leaves were young at the time of inoculation, but quickly took on the character of old leaves, because

the plants were large and the volume of soil small. The strain of *N. rustica* used by Holmes has not been studied by the writer. It may not develop a systemic disease. Localization does not necessarily accompany local necrosis in this species, however.

DISCUSSION

These studies of localization in *Nicotiana* species present evidence that plants on which necrotic spotting develops after inoculation are not always "able to restrain tobacco-mosaic virus from producing a systemic infection." Necrotic spotting is evidence that the cells of the plant are highly sensitive to the virus. Systemic infection in a sensitive or necrotic-spotting plant is accompanied by extensive necrosis of the stem and leaves. This type of mosaic is usually called streak. Systemic infection of chlorotic-spotting plants is of the usual non-necrotic mosaic type. Ambalema was the only variety in which localization of necrotic-spotting strains was complete. In all other varieties and species, even in *N. glutinosa,* systemic infection sometimes occurred. In *N. glutinosa* the invaded cells are so sensitive that the virus usually does not pass beyond the inoculated leaf, altho sometimes streak develops on the stems, or the whole portion of the plant above the inoculated leaf may be invaded and killed. If *N. acuminata* plants are inoculated in the rosette stage, necrosis frequently extends into the stem and causes death of an entire cross section of it. After elongation has taken place, inoculation of leaves causes long stem streaks. In *N. sanderae,* spread of the virus may be extremely slow. Plants inoculated in the rosette stage may develop systemic infection soon after inoculation, or infection may not be evident until stem elongation occurs. If the stem is split, extensive pith and cortex necrosis is found where leaves have wilted and died. In *N. langsdorffii,* infections sometimes become systemic and produce mosaic in all new growth, whereas in other plants, several weeks after their inoculation, the virus spreads slowly upward and causes oak-leaf necrotic patterns along the mid- and lateral veins of successive leaves. Inoculations made from necrotic tissue of the several species studied, to *N. glutinosa* and to Turkish tobacco, gave a

much lower percentage of infection than inoculations from mottled tissue of these species or tobacco. In *N. tabacum* varieties, other than Ambalema, in which local necrotic spotting occurs, systemic infection with white or aucuba mosaic, or other necrotic-spotting strains, is accompanied by severe necrosis of the first invaded leaves, similar to that occurring in *N. rustica* and *N. langsdorffii,* and necrotic streaking of stem both below and above the inoculated leaf. Growing-point leaves develop extensive vein necrosis, but later leaves may be mottled. Midvein and stem streak is rare in *N. tabacum* varieties in which local necrotic spotting does not occur. Necrotic spotting in all species of *Nicotiana* studied is accompanied by a high degree of sensitivity of the cells to the mosaic virus, with the result that invaded cells are frequently killed, and the progress of the virus is retarded. Therefore, the degree of sensitiveness of the species or variety to the virus will determine the extent to which so-called localization may be expected. For example, localization is usual in *N. glutinosa,* much less usual in necrotic-spotting varieties of *N. tabacum,* and infrequent in F_1 hybrids in which a chlorotic-spotting variety of *N. tabacum* is crossed with a necrotic spotting variety. The difference between necrotic spotting and chlorotic spotting varieties seems not unlike that exhibited by a variety of wheat highly resistant to rust, in which cells in contact with the pathogen are quickly killed, and a moderately susceptible variety in which the parasitized cells are not greatly injured by the parasite. There is this difference, however, that in sensitive tobacco varieties the virus may become distributed thruout the plant and virtually destroy it.

The age of inoculated leaves seems to be a determining factor in localizing the virus. Often the local lesions on young leaves consist of chlorotic areas or necrotic dots surrounded by necrotic rings. Inoculations on older leaves of any of the species studied produced more prominent necrotic spots than on young ones, and showed a greater tendency toward localization. If plants are not very active vegetatively, systemic infection is less likely to occur.

In the variety, Ambalema, which is more or less resistant to

all strains of the tobacco-mosaic virus studied, the viruses which cause necrosis on inoculated leaves fail to become systemic even in very young plants. Localization of these strains appears to be more complete in this variety than in any of the other species studied, including *N. glutinosa.* Transfer of the necrosis factor from Ambalema to F_1 and F_2 hybrids with non-necrotic varieties, where systemic infection fairly regularly follows necrotic spotting in heterozygous plants proves the presence of the necrosis factor in Ambalema is not the reason for its resistance and that this factor is not completely dominant. In Ambalema, complete localization seems to result from a combination of sensitivity and resistance. In *N. glutinosa, N. acuminata, N. rustica, N. sanderae,* and *N. langsdorffii,* localization appears to be caused entirely by sensitivity of cells to the virus. If the virus is not localized within the inoculated leaves of these species, its spread is accompanied by necrosis. In *N. sanderae* and *N. langsdorffii,* typical mottle mosaic may appear if the young leaves are invaded, which suggests that in these two species and *N. tabacum* it is the older cells which are hypersensitive. Resistance in Ambalema appears to be caused by a condition within the cell which results in slow multiplication of the virus. For example, necrotic spots develop more slowly on inoculated leaves of Ambalema than on any other species or variety studied. Slow multiplication must necessarily mean slow distribution of the virus within the plant, which may, in turn, explain failure of the virus to invade the growing point. Whether there is an inherent incompatability between young cells of Ambalema and the virus cannot be stated.

The contrast between the rapid rate of development of necrotic spots on inoculated leaves of *Nicotiana glutinosa* and the slower development on necrotic-spotting varieties of *N. tabacum,* or, to mention an extreme case, the very slow development on Ambalema, raises a question as to which species furnishes the best medium for multiplication of the virus. Altho Holmes showed a lower concentration of virus in necrotic tissue than in mottled tissue, he did not prove that the cells which become necrotic were a poorer medium for multiplication of the virus.

The element of time should be considered. The extremely rapid development of necrotic spots on *N. glutinosa* suggests very rapid multiplication of the virus, followed by death of the cells. Slow development of necrotic spots in Ambalema suggests much slower multiplication of the virus. The hypothesis that cells of necrotic-spotting plants provide a better medium for multiplication of virus than non-spotting or slow-spotting plants, and its corollary that necrotic-spotting viruses multiply more rapidly than non-spotting viruses, receives support from a series of inoculation experiments to be described. Seven necrotic-spotting hybrids of Ambalema with chlorotic-spotting varieties of White Burley were inoculated with ten different tobacco-mosaic viruses. A record was kept of the time required following inoculation, for first appearance of systemic symptoms. The average time for fourteen plants inoculated with white or aucuba mosaics (necrotic-spotting) was five and a half days, the average time for fourteen plants inoculated with a green isolant of white, or a yellow mosaic (non-spotting) was seven and a half days, while for 7 plants inoculated with a ring mosaic (non-spotting) the time was nine and a half days. This hypothesis receives further support from the fact that in F_2 hybrids of Ambalema with non-necrotic-spotting varieties of White Burley inoculated with white mosaic, necrotic-spotting plants, homozygous for necrotic factor, develop local symptoms first; plants heterozygous for this factor develop symptoms next in the form of necrotic rings; and last of all, chlorotic spots develop on the homozygous recessive plants. If this hypothesis is finally proved to be correct, it will furnish a much clearer understanding of the rather complete series of diseases represented by the type of infection caused in *N. glutinosa* by all strains of tobacco mosaic as one extreme, in which complete localization within the leaf usually occurs, and that in Ambalema at the other extreme, which shows a high degree of resistance to spread of most strains of tobacco-mosaic viruses, usually unaccompanied by any visible symptoms of infection. A combination in an *N. tabacum* variety of a sensitivity factor comparable to that in *N. glutinosa* (one sensitive to all strains of tobacco-mosaic virus) and resistance of the type

found in Ambalema should result in complete freedom from mosaic.

Local necrotic spotting, sometimes accompanied by localization of the virus, is not confined to the tobacco-mosaic virus. The ring-spot virus quite often causes local spotting and fails to become systemic (8). Many half-grown plants have been inoculated in the field with ring-spot, but very few developed systemic infection. In the greenhouse, if inoculated plants are somewhat starved, or if the inoculated leaves are old, the virus does not become systemic.

Spotted wilt causes local necrotic spots in tobacco. However, if only the older leaves are inoculated, the virus seems to be come localized in the spots, and systemic infection frequently fails to develop. Occasionally, systemic infection appears only after a very long period (sixty-three days or more). Non-development of systemic infection is observed more often in older or less vigorous plants (1). However, if, after weeks of growth, a plant in which systemic infection did not occur is cut back to the bud at the base of an inoculated leaf, the lateral shoot forced into growth may develop a severe necrotic systematic streak disease. There seems to be ample evidence of slow movement of the virus thru plants in which systemic infection does not immediately occur. The virus is not localized, but its spread is so slow that the growing point of the plant may never be invaded.

The different parts of a tobacco plant may be classified as to their resistance to the mosaic virus. The meristematic tissue of the growing point appears to be nearly immune; the cells of young leaves appear to be quite tolerant and are rather rapidly invaded by the virus. The cells of older leaves are more sensitive to the virus (more easily injured), as shown by certain mosaic strains which cause local necrotic spots in older leaves more quickly than in young ones, and by mosaic burning which occurs in large, recently expanded leaves as they are invaded by certain strains of the virus. Young leaves of a necrotic-spotting variety inoculated with a necrosis-producing virus may develop only chlorotic spots; slightly older leaves develop necrotic rings;

and still older leaves develop necrotic spots at the points of entrance of the virus, all of which indicates an increasing sensitivity of the cells as they age.

SUMMARY

Several different strains of tobacco-mosaic virus collected in Kentucky cause necrotic spotting in certain tobacco varieties. The common tobacco-mosaic viruses are nearly always of the non-necrotic type. The necrotic spotting property may be lost from a necrotic-spotting virus. The majority of varieties of tobacco grown in Kentucky develop necrotic spots when inoculated with white or aucuba virus. Localization of white mosaic occurred in the inoculated leaf in 40 percent of 130 necrotic-spotting plants growing in the field. Systemic infection of the remainder was accompanied by extreme necrosis. In the mosaic-resistant variety, Ambalema, necrotic-spotting viruses appear to be completely localized in the necrotic spots. No sharp line can be drawn between localization, such as occurs in certain bean varieties which develop necrotic spots when inoculated with tobacco-mosaic virus, and the commonly observed systemic infection of tobacco plants with the common tobacco-mosaic viruses. A complete series between these extreme conditions can be demonstrated if a series of *N. glutinosa, N. langsdorffii, N. sanderae, N. rustica* and *N. tabacum* plants of different ages are inoculated with several tobacco-mosaic viruses which differ greatly in symptom expression. In each of the species studied, including *N. tabacum,* necrotic spotting is accompanied by sensitivity of the plant to the virus. High sensitivity of cells causes nearly complete localization of the virus in the inoculated leaf, or a very severe necrotic systemic disease; lower sensitivity causes slower development of local spots and is usually accompanied by a mild necrotic systemic disease. Hybrid plants of *N. tabacum* with one sensitivity factor are less severely injured by a necrotic-spotting virus than plants with two factors. Consequently, the sensitivity factor is not completely dominant. Complete localization of a necrotic spotting mosaic virus did not occur regularly in any of the species studied except in Ambalema. Systemic infection, when it occurred following necrotic

spotting, was accompanied by extensive necrosis. Necrotic-spotting is, therefore, merely an index to the degree of sensitivity of the plant to the virus. If the term "localization" is to be used, it should be reserved for particular instances of necrotic spotting where the virus is confined or localized within the inoculated leaf, but should not be used as a term to indicate local necrotic spotting.

With our present varieties of tobacco, localization cannot be expected to be of value in the control of tobacco mosaic, because the common strains of tobacco mosaic are non-necrotic-spotting strains.

Two types of resistance to the tobacco-mosaic viruses may be recognized in *N. tabacum;* one in which certain strains of the virus are sometimes prevented from causing systemic infection by a high degree of sensitivity of invaded cells, with localization in inoculated leaves, and another of the type of resistance shown by Ambalema to non-necrotic strains of the virus, in which the virus is inhibited from entering the young tissue of the plant. Failure of the tobacco-mosaic virus to enter seeds may be the result of inability of the virus to invade meristematic tissue. The suggestion is made that plants which are most highly sensitive to a virus (necrotic-spotting species or varieties) are those in which the virus multiplies most rapidly (*N. glutinosa,* for instance), and those least sensitive are those in which the virus finds the poorest medium for multiplication (Ambalema, for example). Transfer of resistance of Ambalema, either with or without necrotic spotting, to our commercial tobaccos, should prove a satisfactory and practical solution of the tobacco-mosaic problem.

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